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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

A major problem in breast cancer treatment and the leading cause of mortality is invasion and metastasis of primary breast tumors. The cell type from which the tumor arises may dictate its potential for aggressive behavior. The mammary gland consists of different cell types including the cap cell; a less differentiated, highly proliferative cell basally located in the terminal end bud (TEB) of the murine mammary gland. The TEBs invade the fatty stroma of the pubertal gland establishing the ductal network. These specialized structures are reported to be targets for carcinogen-induced DNA damage. Their human counterparts are called intralobular ducts and are also sites of cancerous lesions. We hypothesize that genetic change specific to the cap cell population of the TEB will lead to aggressive tumors and metastatic disease.

P-cadherin is normally expressed in the cap cells of the TEB and its progenitors. To test whether the less differentiated P-cadherin-positive cells have greater metastatic potential, the neu/HER-2 proto-oncogene will be targeted specifically to the cap cell population using the endogenous P-cadherin promoter. The goal of this research is to determine whether the highly proliferative and invasive cap cell population is a target for metastatic breast cancer.

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Table of Contents

INTRODUCTION:

A major problem in breast cancer treatment and the leading cause of mortality is invasion and metastasis of primary breast tumors. The cell type from which the tumor arises may dictate its potential for aggressive behavior. The mammary gland consists of different cell types including the cap cell; a less differentiated, highly proliferative cell basally located in the terminal end bud (TEB) of the murine mammary gland. The TEBs invade the fatty stroma of the pubertal gland establishing the ductal network. These specialized structures are reported to be targets for carcinogen-induced DNA damage. Their human counterparts are called intralobular ducts and are also sites of cancerous lesions. We hypothesize that genetic change specific to the cap cell population of the TEB will lead to aggressive tumors and metastatic disease.

P-cadherin is normally expressed in the cap cells of the TEB and its progenitors. To test whether the less differentiated P-cadherin-positive cells have greater metastatic potential, the neu/HER-2 proto-oncogene will be targeted specifically to the cap cell population using the endogenous P-cadherin promoter. To avoid any toxic effects of the oncogene prior to mammary gland development, we will utilize an inducible expression system in which transgene expression can be tightly regulated *in vivo*. Tumor development will be examined in these animals and tumor pathology will be compared to human breast tumors as well as transgenic models. The goal of this research is to determine whether the highly proliferative and invasive cap cell population is a target for metastatic breast cancer.

BODY:

An outline of our research accomplishments follows, however this one year Concept Award did not have a Statement of Work.

In order to generate an animal model whereby genes can be induced specifically in a subpopulation of breast cells, homologous recombination in ES cells was utilized to specifically target the P-cadherin gene. The P-cadherin gene is normally expressed specifically in cap cells and its progenitors. To make a targeting construct we isolated a genomic clone containing exon 1/ exon 2 of mouse P-cadherin gene by screening a mouse genomic library with a probe corresponding to the 5' region of the gene. The targeting vector was made as described below. The tetracycline-controlled transcriptional activator (rtTA) is the fusion of mutated tetracycline repressor to the VP16 activation domain of herpes simplex virus. The rtTA cassette containing the SV40 polyadenylation signal was fused to a 4.2 kb mouse P-cadherin genomic fragment including the P-cadherin promoter/5'-UTR up to the translation start and placed upstream of floxed *neo*, a positive selectable marker. Thus, *neo* can be deleted from the targeted allele *in vivo* by breeding rtTA knock-in mice with Cre expressing transgenic mice (Jackson Laboratories) where Cre is under regulation of protamine promoter and is only expressed in sperm. An additional genomic fragment (3.9 kb) located immediately downstream of ATG was placed between *neo* and *tk*, a negative selectable marker.

rtTA binds the Tet-Responsive Element (TRE) and activates transcription in the presence of doxycycline. In order to test our construct, an in vitro reporter assay was performed using epithelial carcinoma cell line A431 known to express P-cadherin. These cells were co-transfected with the targeting construct and pTRE-luc plasmid which contains luciferase gene driven off the TRE/minCMV promoter. Luciferase expression in

the presence of 1μg/ml of doxycyclin was 3.5-fold higher as compared to no induction control. These data suggest that our construct can be used to specifically drive rtTA expression in P-cadherin-expressing cells.

D3 ES cells were electroporated with linearized targeting DNA, selected with G418 and gancyclovir, individual clones were picked up and analyzed by Southern blot hybridization of appropriate DNA samples. BglII digest of wild type DNA yields 7.0 kb band while homologous recombination allele gives 9.4 kb. We have performed four independent experiments with some variation of selection conditions. Totally 1,100 clones were analyzed, 32 of them were correctly targeted, and 5 of them were used to generate chimeric mice by blastocyst injection. All chimeras generated from these ES cells exhibited 40-70% contribution (by analysis of coat color) of donor D3 ES cells. Unfortunately, none of the chimeric males transmitted the recombinant allele through the germ line, suggesting a limited use of our ES cells to generate knockout mice. So, we have changed ES cell line to one isolated by Dr. P. Labosky (University of Pennsylvania), which has a lower passage number. We have also redesigned the targeting construct by replacing negative selection marker of thymidine kinase with diphtheria toxin gene. By using this modified vector we avoid the use of gancyclovir, which potentially increases the level of spontaneous differentiation of ES cells. These TL1 ES cells were correctly targeted with above construct (six clones were identified) and used to generate chimeric mice. Two blastocyst injections have been performed, and we obtained chimeras with 85-100% coat color contribution. These are our best chimeras to date and we will begin breeding for germline transmission of the knock-in allele in the near future.

KEY RESEARCH ACCOMPLISHMENTS:

1. Generation of a targeting vector to introduce the rtTA, transcriptional activator, into the P-cadherin locus.
2. Identification of ES cell clones containing the correct homologous recombination event.
3. Generation of chimeric mice by injection of the targeted ES cell clones into blastocysts.

REPORTABLE OUTCOMES: We developed genetically modified ES cell clones, which are being used to generate a novel animal model for breast cancer.

CONCLUSIONS: It was not possible to completely develop this animal model within the time frame of this one year Concept Award, however we now have the necessary reagents to move this project forward.

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APPENDICES: None

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Glenn Radice, Principal Investigator Melanie Lieberman, Research Assistant